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EXPERIMENTAL ARTICLES

Antagonistic Activity of Lactobacilli Isolated from Natural Ecotopes

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Abstract—Lactobacilli are widely used in silage production, for fermentation of foodstuffs, and as probiotics. Their therapeutic effect in preparations is based on their antagonistic activity against pathogens. In this work, antagonistic activity of *Lactobacillus* strains isolated from silage and fermented plant-derived foodstuffs was studied in order to select the strains promising for industry, agriculture, and medicine. Twenty *Lactobacillus* strains were ranked according to the intensity and rate of acid production and antibiotic resistance. *Lactobacillus* sp. Ca9L was selected as a promising starter culture strain for biotechnology based on the optimal combination of acid production, rate of acidification, and antibiotic resistance.

Keywords: lactobacilli, acid production, antibiotic resistance

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Bacteria of the genus *Lactobacillus* (*L. plantarum*, *L. fermentum*, *L. brevis*, *L. casei*, *L. buchneri*, etc.) represent a large group of plant epiphytic microflora. In decaying plants and silage they play a key role in the fermentation of carbohydrates (Hammes and Hertel, 2009). Fermentation of plant foods (sauerkraut, pickles, etc.) also takes place with the participation of lactobacilli limiting the growth and reproduction of undesirable microorganisms, which prevents spoilage and increases the shelf life of foodstuffs (Caplice and Fitzgerald, 1999).

During the development of microbial communities in natural ecological niches bacteriostatic activity of lactobacilli is due to their ability to synthesize antagonistic factors. Usually, they are organic acids produced via or during lactic acid fermentation (mainly lactate). Besides, some species, in particular, heterofermentative (*L. fermentum*, *L. buchneri*, *L. brevis*, *L. reuteri*, *L. plantarum*, etc.) form acetic, succinic, and pyrrolidone-5-carboxylic acids (de Vries et al., 1970; Kaneuchi et al., 1988; Yang et al., 1997). Through the pH decrease in the environment, these lactobacillar products of metabolism inhibit the growth of other microorganisms without causing harmful effects on acid producers themselves (Hütt et al., 2006). Apart from nonspecific acidification of the environment, some *Lactobacillus* species produce a number of bacteriocins with a wide range of targets of their antimicrobial action, including staphylococci, enterobacteria (*Klebsiella, Enterobacter, Serratia, Proteus, Salmonella, Escherichia,* and *Citrobacter*), clostridia (*Clostridium difficile*), listeria (*Listeria monocytogenes*), *Helicobacter pylori*, and others (Servin, 2004). Therefore, *Lactobacillus* strains are used in probiotic preparations for normalization of human intestinal microflora (Liévin-Le Moal and Servin, 2014).

On the other hand, lactobacilli, like all other microorganisms, possess an evolutionary acquired resistance to the antibiotic compounds synthesized by other members of the microbial community, in particular, *Streptomyces*, bacilli, and micromycetes. Inherent antibiotic resistance is due to genetic determinants which are responsible for the degradation of the antibiotic molecules, modification of its target, or release of the antibiotic from the cells by the efflux systems (Blair et al., 2015). Discovery of the dualistic nature of antibiotics resulted in reconsideration of their functional role in the development of microbial communities in natural systems and in symbiosis (Aminov and Mackie, 2001; Aminov, 2009). In high concentrations, in which they are never found in natural systems, antibiotics have a microbicidal function while in low concentrations they are signal substances (Davies, 2006; Fajardo and Martínez, 2008; Kozhevin et al., 2014).

Bacteriostatic activity of the acids produced by lactobacilli has recently been given a great attention as an essential biotechnological characteristic. As for the antibiotic resistance of lactobacilli, this criterion is currently being disputed for probiotic strains. On the one hand, only antibiotic-resistant lactobacilli will survive during antibiotic therapy (Bruslik et al., 2015). On the other hand, there is a great risk of transmission of genetic determinants of antibiotic resistance to pathogenic bacteria (Gevers et al., 2003; Mathur and Singh, 2005).

The goal of the present work was to characterize such properties of natural strains of lactobacilli as their antibiotic resistance and the level of acid production